



Gp/1632

Practitioner's Docket No. 1822/117

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nissim Benvenisty

Application No.: 09/995,452

Group No.: 1632

Filed: 11/27/2001

Examiner: Ton, Thaian N.

For: Transfection of Human Embryonic Stem Cells

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

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AMENDMENT TRANSMITTAL

1. Transmitted herewith is an amendment for this application.

STATUS

2. Applicant is other than a small entity.

EXTENSION OF TERM

3. The proceedings herein are for a patent application and the provisions of 37 C.F.R. 1.136 apply. Applicant petitions for an extension of time under 37 C.F.R. 1.136 (fees: 37 C.F.R. 1.17(a)(1)-(4)) for one month:

Fee: \$110.00

CERTIFICATION UNDER 37 C.F.R. §§ 1.8(a) and 1.10\*

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Date: June 2, 2003

\* Only the date of filing (§ 1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under § 1.8 continues to be taken into account in determining timeliness. See § 1.703(f). Consider "Express Mail Post Office to Addressee" (§ 1.10) or facsimile transmission (§ 1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.

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## FEE FOR CLAIMS

4. The fee for claims (37 C.F.R. 1.16(b)-(d)) has been calculated as shown below:

	(Col. 1)	(Col. 2)	(Col. 3)	OTHER THAN A SMALL ENTITY				
	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA			RATE		ADDIT. FEE
TOTAL	20	- 20	= 0	x	\$	18.00	= \$	0.00
INDEP.	3	- 3	= 0	x	\$	84.00	= \$	0.00
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM				+	\$	0.00	= \$	0.00
TOTAL ADDIT. FEE								\$ 0.00

No additional fee for claims is required.

## FEE PAYMENT

5. Authorization is hereby made to charge the amount of \$110.00 to Deposit Account No. 19-4972.

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## FEE DEFICIENCY

6. An additional extension and/or fee is required, charge Account No. 1904972.

An additional fee for claims is required, charge Account No. 19-4972.

Date: June 2, 2003



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Benvenisty et al.

Atty Dkt: 1822/117

Serial No: 09/995,452

Art Unit: 1632

Date Filed: November 27, 2001

Examiner: Ton, Thaian N.


Invention: **Transfection of Human  
Embryonic Stem Cells**

Date: June 2, 2003

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Timothy M. Murphy #13

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Alexandria, VA 22313-1450

ELECTION

Dear Sir:

The Applicants thank the Examiner for the action mailed April 2, 2003. Applicants elect group VII; however, the Applicants traverse the restriction and request examination of claims 1-17, 36, and 48-56. In particular, the Applicants traverse the restriction between groups I (claims 1-17) and VII (claims 36, 48-56), wherein group I is drawn to methods of altering gene expression in a population of human embryonic stem cells, and group VII is drawn to substantially pure and reagent human embryonic stem cell populations.

The inventions embodied by groups I and VII are not distinct because substantially pure or reagent human embryonic stem cell populations cannot be made by calcium chloride transfection, as maintained by the action. Though calcium chloride transfection is a known technique for transferring DNA in some cell types, the

effectiveness of the technique to transfer DNA varies greatly depending upon the cell type. As such, the ability of calcium chloride transfection to introduce exogenous DNA into human embryonic stem cells cannot be inferred by the technique's success in other types of cells, such as Escherichia coli.

As well, Taketo has shown that electroporation is a superior method to transfect DNA in Escherichia coli in comparison to calcium chloride transfection (the Taketo reference abstract is attached). Since Fig. 1 of the application shows electroporation to be an order of magnitude less efficient for delivering DNA into human embryonic cells, calcium chloride transfection is likely to be far less efficient than the methods revealed by the pending application. Thus, the production of a substantially pure or reagent human embryonic stem cell population would not be feasible.

As such, calcium chloride transfection cannot provide substantially pure or reagent human embryonic stem cell populations as required by claims 36, 48-56. Thus, it is not seen how the cell populations as claimed by group VII can be made by another process, and the Applicants submit that the claims of groups I and VII are accordingly not sufficiently distinct as to warrant the restriction. The Applicants, therefore, respectfully request examination of claims 1-17, 36, 48-56.

In addition, the Applicants reserve the right to prosecute any non-elected groups in subsequent continuation or continuation-in-part applications, including group I if the traverse is unsuccessful.

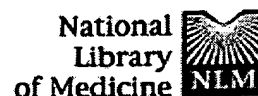
Respectfully submitted,



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## Properties of electroporation-mediated DNA transfer in *Escherichia coli*.

**Taketo A.**

Department of Biochemistry I, Fukui Medical School.

Efficient and reproducible DNA-transfection was attained in *E. coli*, by electroporation. The yield of the transfectants was affected by pretreatment of the recipient cells as well as by the composition of the electroporation medium. Using a single pulse procedure, relationships among the electrical parameters, the transfection efficiency, and the cellular viability were investigated in 10 mM Tris-HCl buffer (pH 7.5) containing 5% sucrose. Certain sodium salts (e.g., citrate, phosphate, and sulfate) were promotive, whereas Mg<sup>2+</sup>, DEAE-dextran, and polyvinylpyrrolidone were inhibitory to the transfection. Heterologous nucleic acids (native DNA, denatured DNA, and tRNA) exerted only a marginal effect on transfection with a viral replicative-form DNA. The efficiency of DNA transfer was affected by culture conditions, and bacteria grown at a higher temperature were more competent. The electroporation system was more efficient than an improved CaCl<sub>2</sub> method, not only in transfection with viral single- and double-stranded DNAs, but also in transformation with plasmid DNAs.

PMID: 2666408 [PubMed - indexed for MEDLINE]

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